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Amphetamines: Correlation of Activity with Stability of Molecular Complexes

(chlorpromazine/Shiff base/serotonin/association constants/trimethoxyamphetamines)

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ABSTRACT **Molecular complexes have been prepared from several biologically active methoxyamphetamines and 1,4-dinitrobenzene. The association constants of these complexes, determined by nuclear magnetic resonance measurements, correlate linearly with the threshold hallucinogenic dose in humans.**

It has been proposed (1) that the mechanism of action of chlorpromazine and other psychotropic drugs, mainly those with aromatic rings, may in some way be due to the electronic-donating ability of these compounds. Attempts to correlate this ability with psychotropic activity (2, 3) have used the calculated energy of the highest filled molecular orbital. Similar correlations have been made (4) with spectral fluorescence intensities of amphetamines. Correlation has also been proposed (5) based on electronic energies valid only for those compounds that fulfilled whatever steric conditions may be required for psychotropic activity. Potency might then be related to electronic energy for a compound or family of compounds satisfying such steric requirements. Such attempted correlations imply the belief that the π-electron donating ability of the amphetamine derivatives may be a determinant of hallucinogenic activity, the amphetamine presumably forming with the brain receptor a weakly bonded, reversible π-molecular complex that gives rise to hallucinations. It has been suggested (6) that the attachment of serotonin at acceptor sites in the brain is by the formation of a weak reversible complex between the indole ring of the serotonin with one site and the formation of a Schiff base between the ethylamine residue of serotonin and an appropriate carbonyl residue at the other site. We believe that the attachment of amphetamines at receptor sites in the brain might be through the same mechanism. It would be of interest to know something of the ability of amphetamines to form π-molecular complexes with electron acceptors (7). Association constants, a direct measure of degree of binding of these molecular complexes, have been determined to see if there exists any correlation with their biological activity.

A number of acceptors were considered for this study. A π-electron acceptor, 1,4-dinitrobenzene, that has been used as the electron acceptor for the various psychoactive phenothiazines (8) was used in this study as an acceptor for several biologically active methoxy-substituted amphetamines. Acceptors stronger than dinitrobenzene, such as tetracyanoethylene and 1,3,5-trinitrobenzene, were tested. An irreversible chemical reaction occurred with these, probably through the amino group (9) of the amphetamine. The nuclear magnetic resonance (NMR) spectrum of 1,4-dinitrobenzene in carbon tetrachloride shows a single sharp line at 8.398 ppm relative to tetramethylsilane. 1,4-Dinitrobenzene is an experimentally convenient acceptor for this study.

The NMR method described by Foster (10) was used to determine the association constants. For a series of solutions where $[D]_0 \gg [A]_0$

$$\Delta^A_{obs}/[D]_0 = -\Delta^A_{obs}K + \Delta^A_{DA}K,$$

where $[D]_0$ and $[A]_0$ are the initial concentration of donor (amphetamine) and acceptor (dinitrobenzene), respectively, K is the association constant for the complex, Δ^A_{obs} is the observed chemical shift of acceptor protons in the presence of various excess concentrations of donor, relative to the chemical shift of the protons in the absence of donor, and Δ^A_{DA} is the chemical shift of the measured protons in the ac-

TABLE 1. *Relative chemical shifts for pure complexes and calculated K values as compared with hallucinogenic activity in man*

Donor	Δ^A_{AD} (Hz)	K (kg/mole)	Hallucinogenic activity*
d-Amphetamine	50.00	0.55	0†
l-Amphetamine	50.74	0.54	0†
2-Methoxyamphetamine	43.33	0.83	?
3-Methoxyamphetamine	43.72	0.96	?
4-Methoxyamphetamine	40.20	1.02	5
2,3-Dimethoxyamphetamine	28.56	1.31	?
2,4-Dimethoxyamphetamine	39.59	1.32	5
2,5-Dimethoxyamphetamine	35.46	1.39	8
2,6-Dimethoxyamphetamine	41.67	1.49	?
3,4-Dimethoxyamphetamine	29.60	1.95	<1
3,4-Methylenedioxyamphetamine	35.60	0.68	3
3,5-Dimethoxyamphetamine	35.41	1.47	?
2,3,4-Trimethoxyamphetamine	16.00	1.70	<2
2,3,6-Trimethoxyamphetamine	22.70	2.00	13 (<10)‡
2,4,5-Trimethoxyamphetamine	27.60	2.43	17
2,4,6-Trimethoxyamphetamine	37.23	2.09	10 (12)‡
3,4,5-Trimethoxyamphetamine	22.80	3.14	2

* Mescaline unit. Data from Shulgin *et al.* (11).

† Zero hallucinogenic activity for amphetamine has been reported (3, 4).

‡ The activity has been reported by Kalbhen (12).

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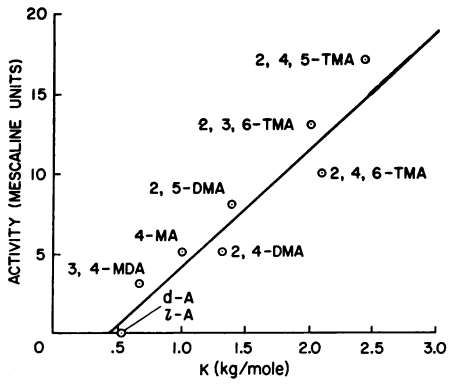

FIG. 1. Correlation between hallucinogenic activity of amphetamine derivatives in humans (mescaline units) and association constant (kg/mol) for several amphetamine-1,4-dinitrobenzene complexes. *A*, amphetamine; *MA*, methoxyamphetamine; *DMA*, dimethoxyamphetamine; *TMA*, trimethoxyamphetamine; *MDA*, methylenedioxyamphetamine.

ceptor moiety for the pure complex, relative to the chemical shift for the pure acceptor protons. A plot of $\Delta^{A}_{obs}/[D]_0$ against Δ^{A}_{obs} is linear, with gradient $-K$ in kg/mol. All NMR measurements were made in carbon tetrachloride solution with a Varian HA-100D spectrometer with a probe at 30.8°. Line positions of the proton resonance of the acceptor molecule were measured relative to tetramethylsilane as an internal reference. Each position was determined six times with a frequency counter to an accuracy of ±0.1 Hz.

The results of relative chemical shifts for pure complexes, the association constants, and the hallucinogenic activity in humans are presented in Table 1. The data show that the unsubstituted amphetamine is a weak donor. The mono- and di-substituted amphetamines have about equal donor strength as *N,N*-dimethylaniline and the phenothiazines, respectively (8). The trimethoxyamphetamines are even stronger complexing agents. This result follows from the fact that as the methoxy group is an electron-releasing group, the more substituent groups on the aromatic ring, the stronger is the electron-donating ability and the higher is the association constant. A correlation plot between the hallucinogenic activity expressed in mescaline units, as measured by Shulgin *et al.* (11), and the association constants, as measured here, is shown in Fig. 1. The best fit by the least-squares method, excluding 3,4-dimethoxyamphetamine, 2,3,4-trimethoxyamphetamine, and 3,4,5-trimethoxyamphetamine is expressed by

$$\text{Activity} = -3.798 + 7.918K$$

The correlation of these data is significant ($F = 101.03$, $\rho = 0.97$, and the 95% confidence bound is 0.74, 0.99). If the results for 3,4-dimethoxyamphetamine, 2,3,4-trimethoxyamphetamine, and 3,4,5-trimethoxyamphetamine are included, the correlation is not significant. The lack of correlation for 3,4-dimethoxyamphetamine, 2,3,4-trimethoxyamphetamine, and 3,4,5-trimethoxyamphetamine might be due to the steric characteristics of 1,4-dinitrobenzene different from those of a physiological receptor site. It seems more likely that these three compounds fit the conformation geometry of 1,4-dinitrobenzene better than they fit the biological receptor. This would account for the observed high K values at low activity. The significant correlation for several amphetamines implies that molecular complex formation may be one of the important factors for the hallucinogenic activity.

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